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Processes structuring macrophyte metacommunities in Mediterranean ponds: combining novel methods to disentangle the role of dispersal limitation, species sorting and spatial scales

Running title: Drivers of macrophyte metacommunities

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ABSTRACT

Aim: Metacommunity ecology is a vibrant area of research that has received increased attention in recent years, since it provides a framework to assess the underlying dispersal- and niche- based processes that create non-random and ecologically meaningful patterns in species assemblages across the landscape. Here we set out to test for the role of dispersal limitation, species sorting and shared effects in the assembly of pond macrophyte metacommunities across an extensive area within the Iberian Plateau, and to identify which traits, environmental variables and spatial scales are driving local community structure.

Location: Northwestern Spain (Iberian Plateau).

Taxon: Pond macrophytes.

Methods: We established a novel combination of robust methods capable of identifying the processes and most important landscape scales involved in the assembly of communities. We used metacommunity assembly modelling and multivariate multiscale codependence analysis to first estimate the relative importance of spatial and environmental effects on community structure, and then to identify significant trait-environment relationships and spatial scales.

Results: Analyses showed that the greatest effects were seen for the spatial and mixed spatial and niche-based scenarios, particularly among wind-dispersed species. Thus, dispersal limitation interfered with species sorting in determining assemblage structure by hindering species' tracking of local environmental conditions. After accounting for this, the metacommunity assembly model revealed that species' traits were involved in determining abundance structure. Multiscale codependence analysis identified the main trait-environment relationships (and spatial scales) as fruit size-nutrient status (~300 km) and growth form-mean pond depth (~250 km).

Main conclusions: Our study suggests that dispersal limitation acted in concert with species sorting to influence the community assembly processes underlying selection for particular traits in functional niche space. Accordingly, we emphasize the need to go beyond the traditional taxonomic-based analyses of community composition and the predominant thinking of considering spatial and environmental processes as two alternative and mutually exclusive scenarios of community assembly.

Keywords: aquatic plants, ecological modelling, functional traits**,** MAM, mMCA, patch dynamics

1. INTRODUCTION

Metacommunity ecology provides a framework to assess the underlying processes that create non-random and ecologically meaningful patterns in species distribution across the landscape (Capers, Selsky, & Bugbee, 2010; Heino et al., 2015a). The metacommunity concept (i.e. a set of sites connected through dispersal) has attracted much interest over the last two decades (e.g. Leibold et al., 2004; Logue, Mouquet, Peter, & Hillebrand, 2011) since it integrates the interplay between spatial processes and local niche-based forces as drivers of community assembly (Holyoak, Leibold, & Holt, 2005). Regional variation in the environment may influence community assembly when dispersal rates allow species to reach suitable habitat patches, resulting in communities controlled by species sorting and niche differentiation (Capers et al., 2010). Conversely, spatial processes may hinder species from tracking environmental variation when dispersal rates are low, constraining species to fewer habitat patches and producing a close relationship between geographic isolation and community structure (Alahuhta, 2015). However, recent theoretical and empirical research (reviewed in Brown, Sokol, Skelton, & Tornwall, 2017) recognizes that the mechanisms represented by metacommunity theory must go beyond the historically predominant thinking of considering dispersal limitation and environmental filtering as two alternative and mutually exclusive scenarios of community assembly (Meynard et al., 2013). Rather, Leibold et al. (2004) and Cottenie (2005) emphasized that metacommunity theory embraces a multidimensional continuum of community assembly dynamics spanning from scenarios dominated by spatial processes to scenarios influenced primarily by species sorting.

In the freshwater realm, studies focusing on a variety of biological groups, from protists to vertebrate metazoans (e.g. Landeiro, Bini, Melo, Pes, & Magnusson, 2012; Heino & Tolonen, 2017), suggest that metacommunities are often structured by a combination of dispersal limitation and species sorting, yet their relative importance varies among different organisms, regions and spatial scales (Heino et al., 2015b). However, the role of niche- and dispersal-based dynamics on aquatic macrophytes of lentic waterbodies is still largely unknown (see Capers et al., 2010; Alahuhta & Heino, 2013; Alahuhta, Johnson, Olker, & Heino, 2014), and most available data come from temperate and boreal deep lakes (e.g. Alahuhta & Heino, 2013; Alahuhta et al., 2014; Alahuhta, Hellsten, Kuoppala, & Riihimäki, 2018). To our knowledge, most previous studies in Mediterranean pond environments have addressed compositional variation in macrophyte assemblages from a local perspective (e.g. Fernández-Aláez, Fernández-Aláez, García-Criado, & García-Girón, 2018; García-Girón, Fernández-Aláez, Fernández-Aláez, & Nistal-García, 2018), thus overlooking the role of dispersal limitation and species sorting in metacommunity dynamics at regional scales. Consequently, there is clearly a need to identify community assembly processes underlying macrophyte biodiversity patterns in Mediterranean landscapes, where habitat fragmentation may modify the dominance of niche- and dispersal- based mechanisms in explaining species distribution (Gallego et al., 2014). Since extensive Mediterranean environments are found in several locations worldwide

and are relatively sensitive to climate and land-use change (Peña-Ortiz, Barriopedro, & García-Herrera, 2015), addressing this knowledge gap has important, widespread implications.

An exciting challenge for metacommunity ecology is to refine statistical tools to identify if community structure along environmental and spatial gradients is consistently associated with selection for particular traits in functional niche space (e.g. De Bie et al., 2012; Brown et al., 2018). A related issue is how best to assess the common assumption that the species making up the assemblage are equivalent in their dispersal biology (Heino et al., 2015b). In this vein, an increasingly promising deconstructive approach is to divide species based on dispersal mode groups (e.g. Cottenie, 2005; Heino, 2013). Although the mechanisms of mobility in aquatic plants (i.e. anemochory, hydrochory, zoochory and autochory) are not clearly defined (Soomers et al., 2013), the premise is that spatial effects are likely to be of major concern for those species that strongly rely on hydrological connections between habitat patches (De Bie et al., 2012). Progress in this area of research may help in elucidating patterns of species diversity that would otherwise be missed in the traditional taxonomic-based analyses of community composition (Heino, 2011).

The standard strategy to investigating metacommunity processes is to perform community variation partitioning using constrained ordination to evaluate the unique and shared effects of spatial and environmental sets of variables (Borcard, Legendre, & Drapeau, 1992). Such an approach is highly correlative and was recently shown to result in greatly inflated estimates of the role of species sorting in common cases where the environment and species distributions are spatially structured (Clappe, Dray, & Peres-Neto, 2018). An alternative technique, which models the process of trait-based community selection from probabilistic species pools, represents a more mechanistic approach. The recent application of such an approach to stream macroinvertebrates has been promising (Brown et al., 2018). This metacommunity assembly model (MAM) is built upon on the well-established theory that sees local community composition as the result of filters acting on species' traits (Poff, 1997), requiring no assumptions about which environmental variables to include. MAM produces information on the influence of dispersal limitation, species sorting, and both in combination, as compared to a null model that excludes deterministic processes (Brown et al., 2018).

A further issue with variation partitioning is that, whilst information on the shared effects of spatial and environmental variables is produced, the outputs give no explicit information about the scales at which niche-based processes are likely to act, and which species or traits are involved. Recent advances in multivariate multiscale codependence analysis (mMCA) now allow for the detection of the scales at which environmental drivers influence multi-species communities (Guénard & Legendre, 2018), i.e. the scales associated with environmental filters (*sensu* Poff, 1997). Such information would be highly complementary to MAM by providing explanations for any combined effects of space and the environment.

Using this novel combination of techniques (MAM and mMCA), we aimed to evaluate the role of dispersal limitation, species sorting and combined effects in the assembly of pond macrophyte communities in a vast area (94,226 km^2) from the northern part of the Iberian Plateau. We stratified the analyses by dispersal group (i.e. anemochory, hydrochory, zoochory and autochory) to test whether certain dispersal modes were more likely to confer dispersal limitation. We hypothesized that: (i) species sorting would be interrupted due to the isolation of ponds within the landscape; (ii) hydrochorous and autochorous taxa would be the most limited by dispersal since both mechanisms strongly rely on hydrological connections; and (iii) environmental drivers of community structure would act at relatively large spatial scales as a result of habitat fragmentation in Mediterranean environments.

2. METHODS

2.1 Study area

A total of 51 permanent ponds were selected for study within a heterogeneous and lowland (700-1100 m above sea level) area of approximately 94,000 km^2 in the Duero drainage basin in northwestern Spain (Figure 1). This region has a Mediterranean climate with a wide seasonal variation in temperature and precipitation, since winters are typically cold and wet (average winter temperature of 3.2 °C and mean winter precipitation of 173 mm) and summers are primarily hot and dry (average summer temperature of 18 °C and mean summer precipitation of 84.5 mm; 1976-2015, data provided by the Spanish Met Agency; AEMET - http://www.aemet.es). The predominant land uses in the study area are arable (46.3%), pasture (28.8%) and woodland (20.5%), as well as pine plantations and scrubland (4.4%; García-Girón, Fernández-Aláez, Fernández-Aláez, & Luis, 2018). The majority of ponds studied are fed mostly by groundwater and rainfall and experience a strong reduction in water volume during the summer, ranging between 0.1 and 23 ha in aerial extent and 0.2 and 6.3 m in depth. The study ponds display considerable variability in environmental conditions, including morphometry, nutrient content and mineralization (Table 1).

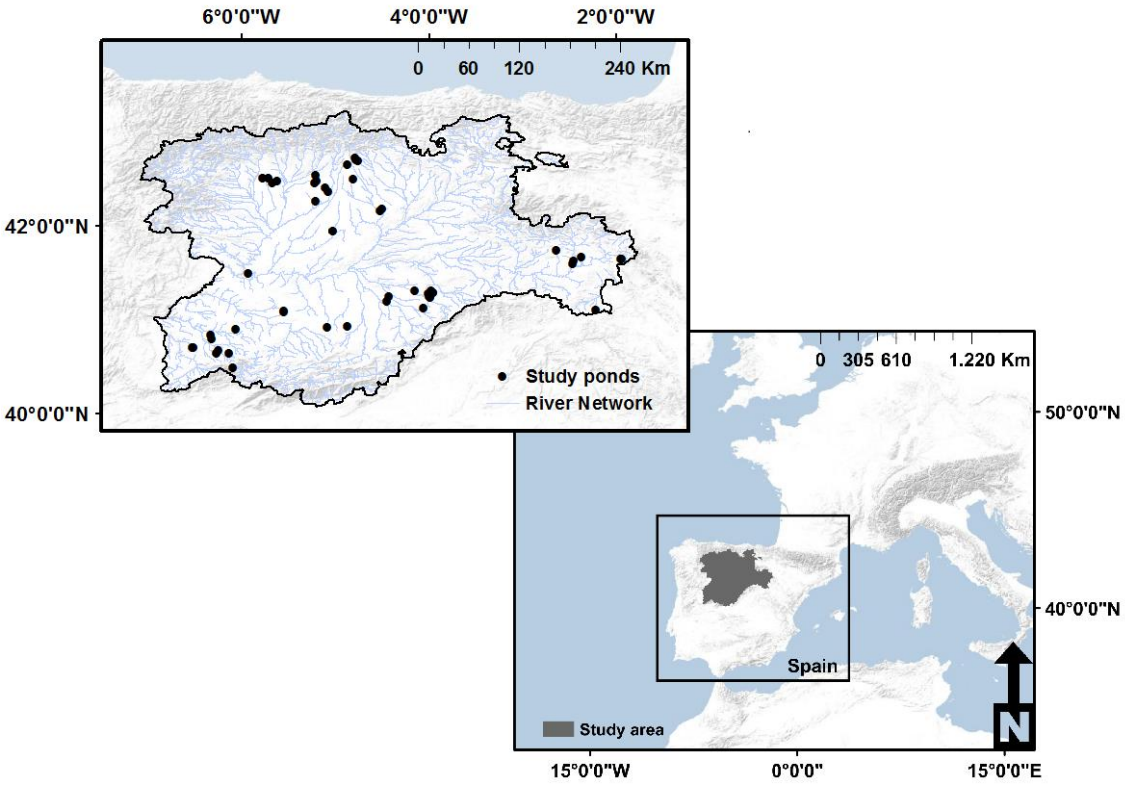

Figure 1. Map of the study area showing the location of the 51 study ponds.

	Minimum	Maximum	Median	Mean
Pond area	0.1	23	2.6	4.3
Mean depth	0.2	6.3	0.7	0.9
pH	6.6	10.3	8.3	8.4
Conductivity	12	1068	215	293
Turbidity	1.4	83.3	9.2	15.7
TN	0.13	5.21	1.49	1.7
NO_3^--N	0	0.25	0.05	0.06
NH_4^+-N	0	0.15	0.01	0.01
TP	19.6	7089.6	118.6	578.8
PO_4^{3-}-P	0.9	6633.8	18.8	578.8
Chla	0.7	362.7	13.9	38.8

Table 1. Summary of the environmental conditions of the 51 study ponds. Pond area (ha), mean depth (m), pH, conductivity (µS cm^{-1}), turbidity (NTU), total nitrogen (TN; mg l^{-1}), nitrate (NO_3^--N; mg l^{-1}), ammonium (NH_4^+-N; mg l^{-1}), total phosphorous (TP; µg l^{-1}), soluble reactive phosphorous (PO_4^{3-}-P; µg l^{-1}) and chlorophyll "*a*" (Chla; mg l^{-1}).

184 **2.2 Field data collection**

185 Each pond was exhaustively surveyed for aquatic macrophytes (emergent, floating-leaved and
186 submerged forms) using profiles in June and July of either 2004 or 2005; hence each pond was
187 sampled once. A profile is defined as a line from one shore to the opposite shore at a right
188 angle to the shoreline with the longest length. The number of profiles for each pond was
189 determined according to the pond area and shoreline complexity (Jensén, 1977), although
190 some corrections were implemented *in-situ* in order to account for the spatial heterogeneity of
191 macrophyte assemblages and the accessibility to the sampling point. Quadrats (0.5 m x 0.5 m)
192 were placed at varying intervals of 0-5 m depending on the homogeneity of the aquatic flora.
193 The total number of profiles and quadrats for each pond was increased regularly with pond
194 area, ranging between 1 and 3 and 5 and 83, respectively (mean density of quadrats per
195 hectare = 5). Percentage coverage of each macrophyte species was estimated in each quadrat
196 as the visual projection of each species in the water column onto the pond surface. Finally,
197 mean coverage of each taxa in a pond was determined as the sum of percent coverages of that
198 species in all quadrats divided by the number of quadrats used in the pond. Nomenclature
199 followed Flora Ibérica (Castroviejo 1986-2012), Fernández-Aláez, Fernández-Aláez, Santiago,
200 Núñez, & Aboal (2012), and Cirujano, Meco, García-Murillo, & Chirino (2014).

201 Pond area (ha) was measured on images available in SIGPAC (the Spanish Geographical
202 Information System for Agricultural Parcels - http://www.sigpac.jcyl.es/visor/), whereas mean
203 depth (m) was determined by measuring depth with calibrated sticks at several sites along
204 profiles within each pond. Several water samples were randomly collected at different depths
205 along a shore-centre transect using a cylindrical corer (diameter = 60 mm, length = 1 m). The
206 number of samples ranged between 3 and 15 depending on the pond area. All samples from
207 each pond were subsequently combined and mixed to form a single composite water sample.
208 A range of environmental variables including pH, conductivity ($\mu S\ cm^{-1}$) and turbidity (FTU)
209 were measured *in-situ* from the composite sample using WTW field probes (Model LF 323) and
210 a portable turbidimeter (Model HACH 2100P). The integrated water samples were preserved at
211 4 °C and then analysed in laboratory to determine total nitrogen (TN; mg l^{-1}), nitrate (NO_3^--N;
212 mg l^{-1}), ammonium (NH^{4+}-N; mg l^{-1}), total phosphorous (TP; $\mu g\ l^{-1}$), orthophosphate (PO_4^{3-}-P; μg
213 l^{-1}) and chlorophyll "*a*" (Chla; mg l^{-1}). Nutrient samples were previously fixed with mercuric
214 chloride ($HgCl_2$) and all analyses followed standard methods (APHA, 1989).

215 **2.3 Data analysis**

216 Using information available in Willby, Abernethy, & Demars (2000), functional traits were
217 selected to provide information on attributes for each macrophyte species that could
218 potentially come under selection by environmental filters. We included fuzzy scores (0 =
219 absence of an attribute, 1 = weak affinity and 2 = strong affinity) for a total of 31 trait
220 modalities subdivided into seven trait categories (Table 2). When no information was available
221 in Willby et al. (2000) (e.g. *Eleocharis palustris* (L.) Roem. & Schult., *Eleocharis multicaulis* (Sm.)

Desv. and *Juncus articulatus* L.), traits were inferred from Castroviejo (1986-2012) and Cirujano et al. (2014). We transformed fuzzy scores according to the established method of Chevenet, Dolédec, & Chessel (1994), whereby trait modalities are expressed as proportions within categories (e.g. growth form) and centred to ensure that the species × trait matrix had equal row and column weights.

Traits	Attributes
Growth forms	Free floating (surface and submerged) and anchored (floating leaved, submerged leaved and emergent leaved)
Mode of reproduction	Rhizomes, fragmentation, budding, turions, stolons, tubers and seeds
Number of reproductive organs per year and individual	Low (<10), medium (10-100), high (100-1000) and very high (>1000)
Perennation	Annual, biennial and perennial
Dispersal vector	Wind, water and animals
Period of production of reproductive organ	Early (March-May), mid (June-July), late (August-September) and very late (post-September)
Fruit size	Small (<1 mm), medium (1-3 mm), large (>3 mm)

Table 2. Choice of traits and their subdivisions into attributes according to Willby et al. (2000).

To analyse the processes structuring pond macrophyte communities, we applied the MAM approach of Brown et al. (2018). In brief, MAM mimics the selection of organisms from the species pool through four alternative components. First, the null component selects taxa from the species pool at random and assigns each taxon its mean abundance across the whole landscape (metacommunity abundance). Second, the dispersal component selects species based on a vector of probability weightings unique to each site, again assigning selected taxa their mean metacommunity abundance. The probability weightings are given by a distance decay function describing the proximity of each species to the site of interest. Third, the trait selection component involves the calculation of community weighted means (CWMs) of sites on the first two axes from a principal coordinate analysis (Laliberté & Legendre, 2010). Taxa are selected from the species pool and assigned abundances iteratively until the CWM of the synthetic community matches that of the observed community within a threshold (±2.5% in our case); another pick of taxa is then taken from the species pool and the process repeated. Finally, the mixed component combines occurrence probabilities from the dispersal component with abundances from the trait selection component. In all cases, the number of taxa selected from the species pool is fixed at the observed species richness for each site. The process is repeated a number of times (k=500 in our case) and the performance of each component reported as the mean and standard deviation of the Bray-Curtis similarity. Using these metrics, we calculated the standardized effect size (SES) as:

$$SES = (\mu_{mod} - \mu_{null})/\sigma_{null}$$

where μ_{mod} is the mean Bray-Curtis similarity from the alternative model components (dispersal, trait selection, mixed), and μ_{null} and σ_{null} the mean and standard deviation of the Bray-Curtis similarity from the null component, respectively. See Brown et al. (2018) for full details of the method. For the purposes of fitting the trait selection component, prior to the analysis we combined functionally identical species. We also stratified the model by dispersal group (i.e. anemochory, hydrochory, zoochory and autochory) to test whether the relative role of habitat isolation and trait filtering in structuring local communities differed by dispersal vector. A species was considered a member of a dispersal group if its fuzzy trait score corresponding to that dispersal group was non-zero.

Finally, we performed mMCA on community (relative abundance) weighted means of transformed trait scores (Guénard & Legendre, 2018). This statistical method estimates spatial structures generated by the joint variation of environment and community composition that are described by an orthonormal set of spatial variables (spatial eigenvectors, also known as Moran's eigenvector maps, MEMs; Dray, Legendre, & Peres-Neto, 2006). We calculated the spatial variables from the irregularly spaced pond locations using the eigenmap function from the `codep´ package in R (Guénard, Legendre, & Pages, 2018). The significance of spatial codependence between macrophyte communities and environmental variables was assessed through a permutation test using the permute.cdp function of the same package. A principal component analysis (PCA) was performed to visualize the relationships between sites, traits and environmental descriptors in niche space. All environmental variables were centred and standardized to unit variance prior to conducting the PCA.

All analyses were performed in R version 3.4.4 (R Core Team, 2018).

3. RESULTS

We identified a total of 58 macrophyte species from 22 different families. A complete list of species and families is provided in Supporting Information Appendix S1. *Eleocharis palustris* (L.) Roem. & Schult. was the most frequent macrophyte species, occurring in 35 ponds (68%). The second and third most frequent species were *Schoenoplectus lacustris* (L.) Palla and *Potamogeton trichoides* Cham. & Schltdl., occurring in 32 (63%) and 25 (49%) ponds, respectively. Species richness among pond communities range from one to 17 (7±4 species per pond). The trait-based analysis showed that the majority of plant species in the study ponds produced a medium (63.8%) to high (70.7%) number of reproductive organs from mid to late summer (~100%), were submerged leaved, perennial and anemochorous (87.9%, 91.4% and 70.1%, respectively), and had medium (62%) to large (29.3%) propagule size (see Table 3).

Traits	Attributes	Short names	Frequency (%)
Growth forms	Free floating	FreeFloatingSurface	10.3
	Floating leaved (anchored)	AnchoredFloatingLeaves	27.6
	Submerged leaved (anchored)	AnchoredSubmergedLeaves	87.9
	Emergent leaved (anchored)	AnchoredEmergentLeaves	63.7
Mode of reproduction	Rhizomes	Rhizome	50
	Fragmentation	Fragmentation	48.3
	Budding	Budding	13.8
	Turions	Turions	15.5
	Stolons	Stolons	46.6
	Tubers	Tubers	3.4
	Seeds	Seeds	100
Number of reproductive organs per year and individual	Low (<10)	LowRepro	10.3
	Medium (10-100)	MediumRepro	63.8
	High (100-1000)	HighRepro	70.7
	Very high (>1000)	VHighRepro	18.9
Perennation	Annual	Annual	25.9
	Biennial or short-lived perennial	BiennialShortPeren	17.2
	Perennial	Perennial	91.4
Dispersal vector	Wind	Anemochory	70.1
	Water	Hydrochory	29.3
	Animals	Zoochory	39.7
	Self	Autochory	64
Period of production of reproductive organ	Early (March-May)	EarlyRepro	32.8
	Mid (June-July)	MidRepro	100
	Late (August-September)	LateRepro	96.6
	Very late (post-September)	VLateRepro	17.2
Fruit size	Small (<1 mm)	SmallFruit	19
	Medium (1-3 mm)	MediumFruit	62
	Large (>3mm)	LargeFruit	29.3

Table 3. Relative frequencies (%) of the morphological and life history traits for the 58 macrophyte species studied. Short names are used in Figure 3.

Under the community assembly model, combining predictions of species´ occurrences and abundances from the dispersal and trait selection components (mixed scenario) improved predictions relative to the pure trait and pure spatial scenarios. The greatest effects were seen for the mixed and pure spatial scenarios when all species were considered together, closely followed by anemochorous, autochorous and zoochorous species (Figure 2). With the exception of water-dispersing macrophytes, the lowest SES values were seen for the pure trait selection model. Hence, weighting each pick from the species pool on the basis of the geographic distance between sites where each species occurred made predicted and observed communities more similar than under the trait selection scenario, in which the composition of artificial communities was constrained by observed CWMs on the first two synthetic trait axes.

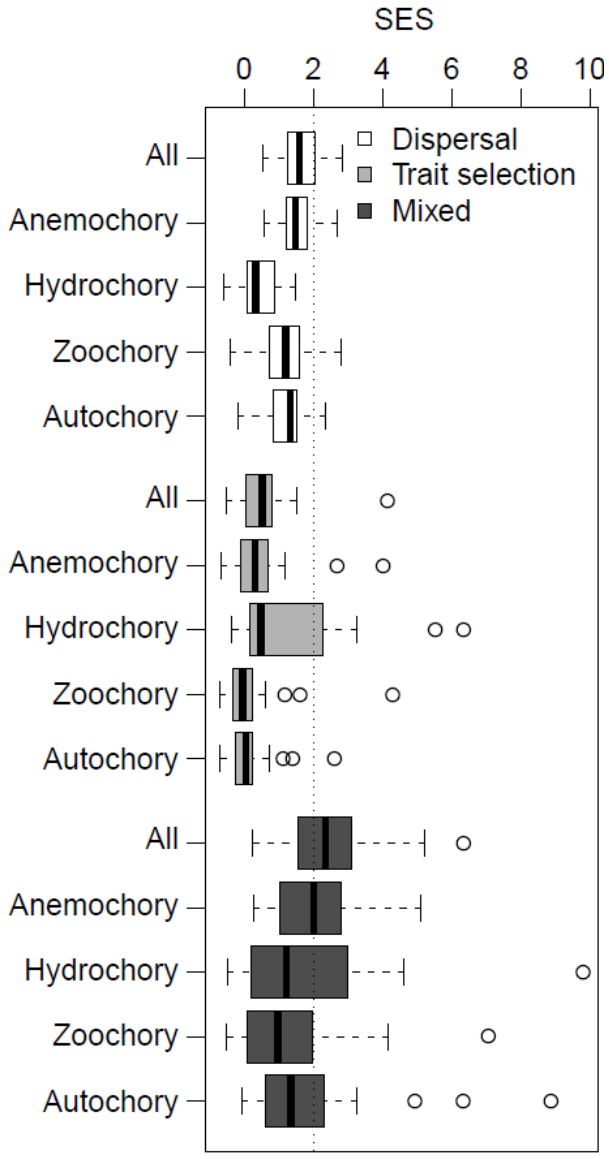

Figure 2. Standardized effect size (SES) for each component of the community assembly model for all species and for species grouped by dispersal mode. The greater the SES values, the better each model component (dispersal, trait selection and mixed) performed compared to

the uniform model. Boxplot bold lines = median; box = interquartile range (IQR); whiskers = maximum and minimum up to 1.5×IQR.

We obtained 48 spatial eigenvectors ranging from the largest potential spatial structures (MEM1) to the smallest (MEM48; Supporting Information Appendix S2). The mMCA application revealed two significant components of the spatial codependence between macrophyte trait structure and environmental variables (Table 4). The strongest component associated total phosphorous with macrophyte trait structure at the scale of the third spatial eigenvector (MEM3). The next strongest component revealed the association of mean depth with trait structure at the scale of MEM11. MEM3 and MEM11 were associated with spatial extents of approximately 300 km and 250 km, respectively (Supporting Information Appendix S2). The first principal component (PCA1) of the macrophyte trait structure (Figure 3) was related to fruit size and total phosphorous. Communities with positive PCA1 loadings tended to be found in P-poor ponds and were dominated by taxa with small and medium fruit sizes and a medium number of reproductive organs per year, whereas negative PCA1 loadings were associated with larger fruit and higher total phosphorous concentrations (Figure 3). PCA2 was related to growth forms and mean depth. Taxa with submerged growth forms had negative loadings and were generally found in shallower ponds, whereas emergent types had positive loadings associated with deeper ponds. The mMCA results indicated a pattern varying radially from a central-south location (MEM3) which linked total phosphorous with trait variation. A more complex pattern was detected linking mean depth with trait structure (MEM11), which separated north-east and southwest zones from a central band (Figure 4). Importantly, the scales identified by mMCA suggest that artefacts arising from the clustered distribution of sampling sites were absent or minimal.

Scale	Environmental variable	$\Phi_{v1,v2}$	v_1	v_2	p
MEM3	**TP**	**61.53**	**32**	**47**	**.005**
MEM11	**Mean depth**	**43.49**	**32**	**46**	**.015**
MEM7	NH_4^+-N	17.62	32	45	0.3

Table 4. Components of the spatial codependence between macrophyte community weighted mean traits and environmental variables assessed by permutation tests. Note that the permutation test adds components until adding further variables does not result in a significant improvement in model fit. Significant components are presented in bold.

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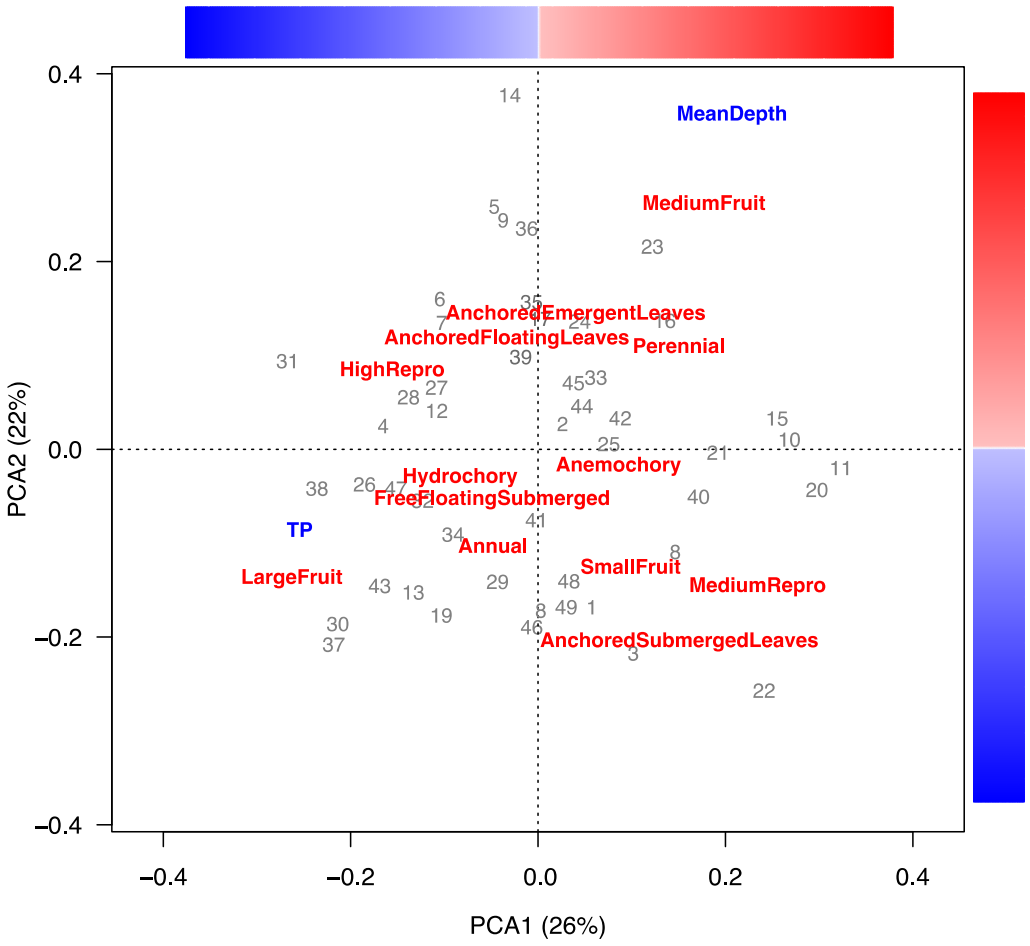

340

341 Figure 3. The first two principal components of the macrophyte trait structure. Sites are
342 labelled using grey numbers, traits (community weighted means) are labelled in red, and
343 environmental variables identified in significant trait-environment relationships by mMCA are
344 labelled in blue (Table 4). To aid interpretation, only those traits with the highest loadings are
345 shown (top 10 on each axis). The colour scales represent values along each principal
346 component as represented in Figure 4.

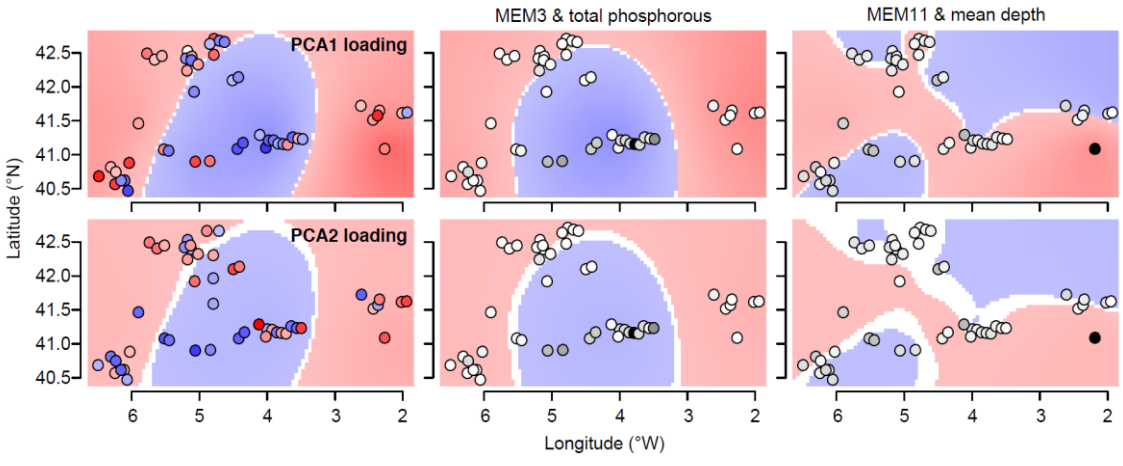

Figure 4. Statistically significant spatial components of codependence between macrophyte trait structure and environmental variables from mMCA. The left panels show sites shaded with colours representing scores from the corresponding principal component (PCA1 and PCA2; Figure 3). Other panels show sites with symbols shaded in greyscale according to the site's value of the environmental variable stated above the map, with background colours corresponding to the spatial structuring variable (MEM) stated above the map (positive values red, negative values blue – see Figure 3). For all panels, background values between sites were obtained from predicted scores of the MEM(s) for single species, which were then projected on the PCA.

4. DISCUSSION

Assessing the relative importance of environmental and spatial processes on community assembly is one of the key approaches for enhancing our basic understanding of metacommunity dynamics (Heino, 2011; Tonkin, Stoll, Jähnig, & Haase, 2016). Importantly, a major appeal of the theory of metacommunity organization is that it potentially offers a predictive framework with which to disentangle systematic relationships between different community assembly scenarios and species´ traits (De Bie et al., 2012; Meynard et al., 2013). Using a combination of data on species´ traits and a robust, probabilistic approach (MAM and mMCA), we assessed the role of dispersal limitation, species sorting and shared effects in the assembly of pond macrophyte metacommunities across an extensive area within the Iberian Plateau, and identified which traits, environmental variables and spatial scales were driving local community structure. We found that the mixed scenario had the greatest standardized effect size, suggesting that dispersal limitation acted in concert with species sorting to influence the structure of local communities. Similarly, our results revealed that nutrient status (total phosphorous) selected for fruit size at the largest extents (~300 km) and mean pond depth selected predominantly for growth forms (submerged and emergent) at a slightly smaller extent (~250 km).

4.1 Drivers of metacommunity structure

Consistent with our first hypothesis and in agreement with a growing number of studies (e.g. Cottenie & De Meester, 2004; Capers et al., 2010; Akasaka & Takamura, 2012; De Bie et al., 2012; Padial et al., 2014), we found that dispersal limitation interfered with species sorting in determining macrophyte community assembly patterns. However, caution should be exercised when comparing the influence of species sorting and dispersal constraints on freshwater macrophytes from one study to another (Shurin, Cottenie, & Hillebrand, 2009). This is because the degree to which dispersal limitation interacts with environmental filtering is likely to vary among different spatial extents of observation (e.g. regional *vs* continental – Alahuhta, Rääpysjärvi, Hellsten, Kuoppala, & Aroviita, 2015 *vs* Viana et al., 2014) and geographic regions

(e.g. Temperate *vs* Mediterranean – Alahuhta, 2015 *vs* this study), with generally stronger dispersal limitation at long rather than short distances (Heino, 2011).

Our results are broadly in line with several studies encompassing a wide variety of organisms (i.e. benthic diatoms, rotifers, cladocerans, macroinvertebrates, molluscs, fish and amphibians), study systems (i.e. wetlands, lakes and streams), and spatial scales (i.e. regional to continental), suggesting that these communities are jointly structured by dispersal limitation and species niche differences (e.g. Pinel-Alloul, Niyonsenga, & Legendre, 1995; Soininen, Lennon, & Hillebrand, 2007; Shurin, Cottenie, & Hillebrand, 2009). Furthermore, our findings agree with Capers et al. (2010), O´Hare, Gunn, Chapman, Dudley, & Purse (2012) and Grimaldo et al. (2016), who recently found that spatial structuring and environmental control together accounted for much of the variation in aquatic plant communities across Connecticut (northeastern US), Scotland and Zambia. By contrast, our results deviate slightly from those found by Alahuhta & Heino (2013) and Alahuhta et al. (2015) in the US state of Minnesota and southern Finland, respectively, suggesting that the relative influence of dispersal- and niche-based processes on macrophyte community assembly is likely to vary rather unpredictably at continental scales.

Our study is the first to illustrate the interaction between spatial and environmental gradients in determining the functional structure of pond macrophytes in Mediterranean landscapes. These findings suggest that there is no strong dichotomy between community assembly scenarios – dispersal limitation and species sorting are extremes along a spectrum of processes underlying observed biodiversity patterns (Heino et al., 2015b). Our results re-emphasize the need to go beyond the traditional view of understanding spatial and environmental processes as two alternative and mutually exclusive scenarios of community assembly and embrace the full power of metacommunity theory (Leibold et al., 2004; Logue et al., 2011; Brown et al., 2017).

4.2 Comparison between dispersal mode groups

Freshwater organisms exhibit a range of dispersal modes and capacities that should be considered when developing a predictive framework for metacommunity dynamics (Heino, 2011). Modern molecular and biogeographical studies (e.g. Capers et al., 2010; Wu, Yu, Wang, Li, & Xu, 2015; Cao, Mei, & Wang, 2017) suggest that some aquatic plants may disperse more or less uniformly at distances up to ~ 200 km, beyond which habitat isolation usually becomes limiting. Our study ponds were largely isolated from each other within a terrestrial matrix (maximum pairwise distance ~ 400 km), so the further apart ponds were, the less likely they were to share a similar species composition. In these kinds of fragmented Mediterranean environments, wind usually plays the primary role for passive dispersers (Coughlan, Kelly, & Jansen, 2017), transporting propagules to other ponds over the landscape. However, contrary to expectations, our results suggest that hydrochorous taxa were the least limited by geographical distances between ponds. This finding is likely to be linked to the existence of

intermittent corridors between ponds via drainage ditches (Junta de Castilla y León, 2001), and agrees with the observation of Soomers et al. (2013), who found that dispersal distances of propagules in fragmented landscapes were many times longer due to hydrochorous dispersal compared to wind dispersal. Since dispersal distances by water are likely to surpass those by wind alone even for typical wind-dispersing macrophyte species (Soomers et al., 2013), anemochorous dispersal might further hinder aquatic plants from reaching new habitat patches when little or no physical connection via flowing water exists between ponds (Boedeltje, Bakker, Ten Brinke, van Groenendael, & Soesbergen, 2004). Given that the majority of plant species in our study ponds were anemochorous and autochorous (see Table 3), it seems reasonable to suppose that macrophyte dispersal may be dependent on dispersal traits, such as propagule size, and the success of dispersal vectors, particularly wind, at overcoming geographic distances and habitat isolation. However, we cannot rule out the degree to which networks of stepping-stone ponds may function as habitat connectivity providers and their implications for species movement among otherwise isolated habitat patches (Saura, Bodin, & Fortin, 2014).

4.3 Species´ traits, environmental drivers and spatial scales

We found evidence for relationships between species´ traits and the spatial scales at which environmental drivers influence community assembly. Based on the mMCA results, we were able to confirm our expectations that macrophyte community trait structure (i.e. fruit size and growth forms) can be affected by environmental variation (i.e. total phosphorous and mean pond depth) via different processes that operate at relatively large spatial scales. Specifically, the role of phosphorous may be interpreted as a relationship between energy-allocation strategies and the availability of a limiting resource (Daoust & Childers, 2004), so that adaptation to increased nutrient levels generally leads to the development of larger vegetative and reproductive structures, particularly fruit size, and a decrease in the biomass of resource-acquiring structures (Li, Werger, de Kroon, During, & Zhong, 2000). The spatial codependence between fruit size and phosphorous at the largest extent is likely to be related to the well-known impact of intensive agriculture on water quality at regional spatial extents (Declerck et al., 2006). Similarly, the relationships between macrophyte growth forms and pond depth in shallow lakes and ponds has already been discussed elsewhere (e.g. Scheffer, 1998), suggesting that this effect is largely due to the attenuation of light at increasing depths which is unfavourable for macrophytes with submerged leaves. Conversely, the potential for phenotypic plasticity in morphological traits (e.g. elongation of stems and increased above-ground biomass) may facilitate the survival of emergent vegetation in deeper, turbid ponds (Coops, van den Brink, & van der Velde, 1996).

4.4 Implications for further studies

Recent recognition of the limitations affecting the traditional approach to assessing community assembly processes called for the development of more sophisticated methods.

Specifically, an analysis of real and simulated community data by Clappe et al. (2018) highlighted the risk of obtaining inflated estimates of species sorting effects from application of the classic variation partitioning framework. They proposed a method whereby estimates of the shared contribution of environmental and spatial effects are adjusted to account for spatial autocorrelation. Whilst this is clearly an advance over the standard variation partitioning approach, we have chosen to apply a combination of methods that are less reliant on correlations between environmental variables and the community matrix. Our approach instead relies on well-established ecological theory (Poff, 1997) that sees local community structure as the result of trait-based filtering of species (i.e. MAM) at different spatial scales (i.e. mMCA). A particular advantage of our approach for future studies in metacommunity ecology is that it simultaneously provides estimates of the relative contributions of spatial and niche-based processes whilst explicitly identifying the traits, environmental variables and spatial scales involved. Such a mechanistic approach may help in elucidating patterns of community assembly that would otherwise be missed, or even misrepresented, under the standard variation partitioning framework.

5. CONCLUSIONS

Our results suggest that dispersal limitation acted in concert with species sorting to influence the community assembly processes underlying observed macrophyte biodiversity patterns. The novel combination of recently developed statistical models, MAM and mMCA, revealed that species' traits were involved in determining abundance structure via two major trait-environment relationships (fruit size-nutrient status and growth form-mean pond depth) that operated over relatively large spatial extents (250-300 km). These results emphasize the need to go beyond the traditional taxonomic-based analyses of community composition and the predominant thinking of considering spatial and environmental processes as two alternative and mutually exclusive scenarios of community assembly. Accordingly, the combination of data on species´ traits and community structure underlies the most promising predictive framework to embrace the full scope of metacommunity theory. Our results support calls for conservation and environmental management to assess community assembly patterns and processes operating at multiple scales across the landscape.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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662 **BIOSKETCH**

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668 and revising the manuscript.